Title: Isolation rearing impairs novel object recognition and attentional set shifting performance in female rats.

Running Title: Effects of isolation rearing on cognition in rats.

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Abstract

Rationale: It has been suggested that the isolation rearing paradigm models certain aspects of schizophrenia symptomology. *Objective*: This study aimed to investigate whether isolation rearing impairs rats' performance in two models of cognition: the novel object recognition and attentional set-shifting tasks, tests of episodic memory and executive function respectively. *Methods*: Two cohorts of female Hooded-Lister rats were used in these experiments. Animals were housed in social isolation or in groups of five from weaning, post-natal day 28. The first cohort was tested in the novel object recognition test with inter-trial intervals (ITI) of 1 min up to 6 hours. The second cohort was trained and tested in the attentional set-shifting task. *Results*: In the novel object recognition test isolates were only able to discriminate between the novel and familiar objects up to 1 hour ITI, whereas socially reared animals remembered the familiar object up to a 4 hour ITI. In the attentional set shifting task isolates were significantly and selectively impaired in the EDS phase of the task ($p<0.01$). *Conclusions*: Rats reared in isolation demonstrate impaired episodic memory in the novel object recognition task and reduced ability to shift attention between stimulus dimensions in the attentional set-shifting task. Since schizophrenic patients show similar deficits in performance in these cognitive domains these data further support isolation rearing as a putative preclinical model of the cognitive deficits associated with schizophrenia.

Keywords Schizophrenia; Attentional set-shifting; Episodic memory; Female rat; Isolation rearing

Introduction

Impaired cognitive function is a key characteristic of schizophrenia (see Sullivan *et al.*, 1994; Elvevag and Goldberg, 2000; Kuperberg and Heckers, 2000 for reviews) and is becoming increasingly important as impaired cognition has been implicated in social and vocational outcome (Marder and Fenton, 2004). These cognitive symptoms can have a great detrimental effect on patients' quality of life (Green, 1996; Green *et al.*, 2000) and can often persist when other symptoms may be improved with antipsychotic treatment (Marder and Fenton, 2004). Both the novel object recognition and attentional set-shifting tasks have been highlighted by the MATRICS initiative as being relevant translational models for studying visual learning and memory and problem solving ability respectively pre-clinically (www.matrics.ucla.edu).

The novel object recognition task (Ennaceur and Delacour, 1988) is a non-spatial test of recognition memory which is ethologically relevant as it relies on the animal's natural tendency to explore novel environments/objects. This model is relevant to the disease since visual recognition memory is impaired in schizophrenic patients (Calkins *et al.,* 2005). The perceptual attentional set-shifting task investigates the ability of a rat to learn a rule and form an attentional set within the same sorting category (intra-dimensional shift - IDS), as well as the ability to shift attentional set between different sorting categories (extra-dimensional shift - EDS) (Birrell and Brown, 2000). It represents a rat analogue of the human Wisconsin Card Sorting Task (WCST, Berg, 1948) and CANTAB ID/ED task (Downes *et al.,* 1989) in which schizophrenic patients exhibit impaired set-shifting (Kolb and Wishaw, 1983; Haut *et al.,* 1996; Pantelis *et al.,* 1999; Tyson *et al.,* 2004).

Early observations of behaviour in rats reared in isolation led to the portrayal of the "isolation syndrome" (Hatch *et al.,* 1965; Sahakian *et al.,* 1977). Rats reared in isolation from weaning until adulthood show several behavioural changes, consistently including increased locomotor activity, anxiogenesis, (Puglisi-Allegra and Oliverio, 1983), enhanced sensitivity to psychoactive drugs such as amphetamine and cocaine (Jones *et al.,* 1990; Smith *et al.,* 1997) and sensorimotor gating deficits as measured by reduced pre-pulse inhibition (PPI) of a startle response (Geyer *et al.,* 1993; Cilia *et al.,* 2001; 2005). However, relatively few reports have investigated the effect of isolation rearing in cognition (Schrijver and Wurbel, 2001; Dalley *et al.,* 2002; Weiss *et al.,* 2004, Li *et al.,* 2007).

The aim of these experiments was to investigate the effects of isolation rearing on cognitive performance in the novel object recognition and attentional set-shifting tasks in female rats. These behavioural tests are of significant importance in modelling cognitive dysfunction associated with schizophrenia, and may be effectively employed to screen novel compounds with the potential to improve such symptoms.

Materials and methods

Subjects

Two cohorts of female hooded-Lister rats (Harlan, UK) were used in these experiments. Both cohorts consisted of ten social and ten isolation reared subjects. Animals weighed 200-240g, were housed in a temperature controlled room (21± 2 °C) at humidity of 45-55% and maintained on a 12/12hour light/dark cycle (lights on at 0700h). All experimental procedures were performed during the light phase. Rats were obtained at 21-23 days post weaning and randomly allocated to one of two housing conditions.

Half were housed in groups of five in clear plastic cages (38 x 59 x 24 cm) and half were housed in individual opaque cages (25 x 35 x 24 cm) for a minimum period of 6weeks before testing. Rats could hear and smell other animals but could not see or come into physical contact with them. Food intake was restricted to 90% of free feeding body weight for cohort 2, while cohort 1 had free access to food. Both cohorts of animals were allowed *ad libitum* access to water. All experimental procedures were carried out in accordance with the Animals (Scientific Procedures) Act UK (1986) and were approved by the University of Bradford ethical review process.

Locomotor activity

The locomotor activity (LMA) response to a novel environment was monitored using automated photocell cages. The movement of each animal was monitored in a Plexiglas chamber (16 x 26 x 19 cm) covered with a compatible Plexiglas lid using AM1052 Activity Monitor (Linton Instrumentation). Counts were recorded using AmLogger software by means of photo beam interruptions within the chamber. Activity was monitored every 5min over a 60-min period and summed to give a total count. Both cohorts of animals were tested in these chambers.

Novel Object Recognition Task

Cohort 1 was tested in the novel object recognition (NOR) task as described by Grayson *et al.* (2007). Following a 3-min habituation session on the day of testing each rat was placed into the NOR chamber and exposed to two identical objects (A1 and A2) for a period of 3 minutes. The objects used were opaque plastic pyramids, small glass jars, cola cans and striped plastic bottles. The heights of the objects were comparable (10 ±

2 cm) and they were heavy enough not to be displaced by the animals, to achieve this, some objects were filled with NaCl. Objects were positioned 6 cm away from the walls of the box, in opposite corners. The rats were then returned to their home cage for an inter-trial interval, the entire box was cleaned, both objects removed and one replaced with an identical familiar copy and one with a novel object. For this study a range of inter-trial intervals (ITIs) were used (1 min, 1, 3.5, 4, 5 and 6 hours). These intervals were chosen based on previous data from our laboratory, which showed that socially housed rats were able to discriminate between the novel and familiar objects up to an ITI of 3 hours (Sutcliffe *et al.,* 2007). It has also been previously shown that the discrimination ability of isolation-reared rats was impaired following an ITI of 1 hour (Bianchi *et al.,* 2006). Subsequently, only socially housed animals were tested at five and six hour ITIs to re-validate their inability to remember the familiar object at these times in this experiment. Repeated testing of animals was necessary due to the limited number of animals with two ITI experiments conducted per week. Following the respective ITI, rats were returned to explore the familiar (A) and novel object (B) in the test box for a 3-min retention trial. The location of the novel object in the retention trial was randomly assigned for each rat using a Gellerman schedule. All experiments were filmed and video recorded for subsequent behavioural analysis by an experimenter blind to the treatments. Locomotor activity was also recorded; this was evaluated by scoring the total number of sectors or line crossings by the animal in both acquisition and retention trials. The exploration time (s) of each object in each trial was recorded manually using two stopwatches and the following factors were calculated.

E1 = the total exploration time of both objects in the acquisition trial ($E_{A1} + E_{A2}$)

E2 = the total exploration time of both objects in the retention trial ($E_A + E_B$).

D1 score = (E_B-E_A) and represents the difference in time spent exploring the novel and familiar objects.

Attentional set-shifting

Cohort 2 were trained and tested in the attentional set-shifting procedure as described in detail in McLean *et al.* (2008), except rats were tested on odour in simple discrimination and digging medium was the parameter used in the extra-dimensional shift. It has been shown that rats find the difficulty of each discrimination change, i.e. medium to odour or odour to medium, equivalent (Birrell and Brown, 2000). In all cases rats were tested in the attentional set-shifting procedure 24 hours after training. The first stage of testing was the simple discrimination (SD), which was identical to the simple discrimination in the training session on the previous day, except new exemplars were used. Testing continued until the rat reached a criterion of six consecutive correct responses. For the compound discrimination (CD) a second dimension was introduced (medium), but the correct and incorrect exemplars remained the same (odour). For the reversals the exemplars and relevant dimensions remained the same (odour) but the rats had to learn that the previously baited odour was now incorrect and the other odour was now the correct one. New exemplars were used for the ID and ED shifts. The specific exemplars used are shown in Table 1. For the ED shift the previously irrelevant parameter (i.e. medium) was now relevant.

Data and statistical analysis

LMA data were analysed by Student's unpaired t-test and were expressed as total counts in a 60-min period. Set-shifting data for total trials to criterion (TTC) and exploration

of both bowls were arcsine transformed and analysed using repeated measures ANOVA with two factors, one within subjects (test: SD, CD, R1, IDS, R2, EDS, R3) and one between subjects (housing: social or isolate) followed by planned comparisons on the predicted means. Data for the novel object task i.e. time at novel versus familiar was also analysed using planned comparisons on the predicted means. All data are presented graphically as observed means with SEMs.

Results

Locomotor activity

Isolation reared rats exhibited a significantly larger locomotor response to a novel environment when compared with socially housed rats in both cohort 1: 1811 ± 149 (social) *cf.* 2620.1 ± 191 (isolate) counts per 60 min ($p<0.05$) and cohort 2: 2247.7 ± 166 (social) *cf.* 3141.9 ± 236 (isolate) counts per 60 min ($p<0.01$). The number of line crossings in the novel object task were consistent at all inter-trial intervals with no significant difference between socials and isolates (data not shown).

Exploratory activity during the acquisition trial of the novel object task

Two socially housed rats (one at 4 h and one at 5 h ITI) and one isolation-reared rat (at 4 h ITI) were excluded from analysis as they failed to move in the box in both the acquisition and retention trials therefore group sizes were 8-10 at each ITI. There was no significant difference in time spent exploring the two identical objects during the acquisition trials at any of the ITIs tested in either socially housed or isolated rats (table 2).

Exploratory activity during the retention trial of the novel object task

Socially housed rats (fig. 1a) explored the novel object more than the familiar object following a 1 minute [$p<0.001$], 1 hour [$p<0.001$], 3.5 hour [$p<0.01$] and 4 hour ITI [$p<0.001$] but not following a 5 [$p=0.69$] or 6 hour ITI [$p= 0.66$]. In contrast, isolation reared rats (fig. 1b) explored the novel object more than the familiar object following only a 1-minute ITI [$p<0.001$] and 1 hour ITI [$p<0.001$] and not following a 3.5 hour ITI [$p=0.86$] and 4 hour ITI [$p=0.49$]. This effect is also seen on the D1 score with isolated rats exhibiting a significantly lower D1 score than socially housed rats at ITIs of 3.5 and 4 hours [$p<0.05$ in both cases, table 3].

Total exploratory activity

Total exploration of both objects by socially housed rats in the acquisition trial was significantly reduced after the 5 hour [$p<0.05$] and 6 hour ITI [$p<0.01$] compared to the 1 min ITI. This was also significantly reduced in isolates during the acquisition trial following a 1 hour [$p<0.05$], 3.5 hour [$p<0.01$] and 4 hour ITI [$p<0.01$] when compared to the 1 min ITI (table 4). This effect was not seen during the retention trial i.e. the total exploration time was not significantly reduced when compared with the 1 min ITI in either socials or isolates.

Attentional set-shifting performance

A total of 17 rats from cohort 2 were successfully trained and tested, rats were excluded from the study due to non-completion of the training within a single session, as rats were required to complete the training simple discriminations within 30 trials. Control rats did not require more trials to reach criterion in the EDS phase compared to the IDS phase; however, isolation-reared rats showed a selective deficit at the EDS stage of the

task requiring significantly more trials to reach criterion than socially-housed rats only at this stage of testing [$p<0.01$; fig. 2]. Isolates also showed the greatest exploration of both bowls before digging when compared to socials in the EDS phase [$p<0.01$; fig. 3].

Discussion

The aim of these experiments was to investigate the effect of isolation rearing on cognitive performance in the novel object recognition and attentional set-shifting tasks in female rats. The results demonstrate that isolation reared rats from both cohorts showed hyperactivity when compared to socially housed controls. Since this is in agreement with previous results in ours (Smith *et al.,* 1997) and many other laboratories (Bakshi and Geyer, 1999; Lapiz *et al.,* 2003; Elliott and Grunberg, 2005; Bianchi *et al.,* 2006) this validated the isolation rearing paradigm used. The principal findings show that isolation reared rats exhibit reduced ability in the novel object recognition task and impaired set-shifting ability in the attentional set-shifting task when compared with socially reared controls. Thus, socially housed animals could discriminate between the novel and familiar objects in the retention trial up to an inter-trial interval of 4 hours, whereas isolates could only discriminate between the novel and familiar objects up to a 1 hour inter-trial interval. In the attentional set shifting task isolates required significantly more trials to reach criterion selectively at the EDS phase of testing, when compared with socially housed animals indicative of a selective deficit in set shifting ability.

Patients with schizophrenia exhibit impairments in face recognition and visual object recognition memory (Calkins *et al.,* 2005). In the current study rats reared in isolation exhibited a similar profile when tested in the novel object recognition

paradigm. It is important to note that there was no significant effect on exploration of the two identical objects in the acquisition trial in either the socials or isolates, but that a selective deficit was observed only in the retention trial. Thus, isolation reared female rats could differentiate between the novel and familiar objects after inter-trial intervals of 1 min and 1 hour but not at 3.5 and 4 hours. This is supported by previous work showing that isolates were able to discriminate a novel object following a delay of 1 minute (Lapiz *et al.,* 2000), but not following a longer ITI (Bianchi *et al.,* 2006) although further investigation would be required to ascertain the exact time at which the isolates lose their ability to discriminate between the novel and familiar objects, by examining a range of ITIs between 1 hour and 3.5 hours. Our results disagree partially with Bianchi and colleagues (2006), as they found isolated male rats could not discriminate between novel and familiar objects after an ITI of 1 hour, whereas after this interval in our isolation-reared rats the ability to differentiate novel from familiar objects remained intact. This may be due to the use of female rats in our study as we have previously shown that females can discriminate between novel and familiar objects following longer ITIs when compared to male rats (Sutcliffe *et al.,* 2007).

Schizophrenic patients also exhibit selective deficits for the ED shift of the ID/ED task and WCST (Haut *et al.,* 1996; Tyson *et al.,* 2004; Jazbec *et al.,* 2007). The data herein show a similar profile in rats reared in isolation. Notably there were no differences between isolates and socials in any other part of the task including reversal learning, showing that reversal learning is not affected by isolation rearing. This is in agreement with previous data from our laboratory in an operant lever pressing task (Abdul-Monim *et al.,* 2006). Our data is also supported by a study using an 8 arm radial maze to assess attentional set-shifting ability showing that isolates performed

similarly in the acquisition and reversal phases but were impaired in attentional shifts (Schrijver and Wurbel, 2001). This may be due to the different regions of the frontal cortex involved in reversal learning and attentional set-shifting. It has been shown that whereas lesions of the orbital prefrontal cortex in rats selectively disrupts reversal learning (McAlonan and Brown, 2003), lesions of the medial frontal cortex (mPFC) produce a selective deficit in the EDS (Birrell and Brown, 2000). Consistent with a selective deficit at the EDS rats reared in isolation have been shown to exhibit structural abnormalities selectively in the mPFC (Silva-Gomez *et al.,* 2003; Day-Wilson *et al.,* 2006). Although a deficit in attentional set-shifting performance in isolation reared rats had previously been described by Schrijver and Wurbel (2001), our study differs in that we employed the Birrell and Brown (2000) method of attentional set-shifting and female, not male rats. Schrijver and Wurbel used an 8-arm radial maze with spatial and non-spatial visual discriminations. An additional interesting finding in our study was that isolates explored both bowls more before making a decision to dig. This was measured by an increased number of visits to both bowls and could suggest that isolates are more indecisive or could be an indication that they cannot remember which is the correct parameter from the discovery trials. We have also shown this effect in female rats treated sub-chronically with the psychotomimetic PCP (McLean *et al.,* 2008). Further investigation is required to elucidate the relevance of this behaviour.

Both forms of cognitive deficit shown in this study could be attributed to structural and neurochemical abnormalities previously demonstrated in rats reared in isolation. Schizophrenic patients exhibit decreased dendritic spine density in both the hippocampus and medial prefrontal cortex (Glantz and Lewis, 2000). These structural alterations are also seen in rats reared in isolation (Varty *et al*., 1999; Silva-Gomez *et*

al., 2003; Day-Wilson *et al.,* 2006) and are regions suggested to be involved in the novel object recognition and attentional set shifting tasks respectively (Clark *et al.,* 2000; Birrel and Brown, 2000). More recently deficits in the novel object recognition task have been associated with structural alterations in hippocampal cytoskeletal microtubules (Bianchi *et al.,* 2006).

In conclusion, rats reared in isolation show deficits in recognition memory and executive function as demonstrated by impaired ability to discriminate between the novel and familiar objects in the retention trial of the novel object recognition test and poor performance in the EDS phase of the attentional set-shifting task. The findings of this study support isolation rearing as a useful model for mimicking cognitive dysfunction with relevance to schizophrenia.

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References

Abdul-Monim Z, Reynolds GP, Neill JC (2006) The effect of atypical and classical antipsychotics on sub-chronic PCP-induced cognitive deficits in a reversal-learning paradigm. Behavioural Brain Research 169: 263-273

Bakshi VP, Geyer MA (1999) Ontogeny of isolation rearing-induced deficits in sensorimotor gating in rats. Physiology and Behaviour 67: 385-392

Berg EA (1948) A simple objective treatment for measuring flexibility in thinking. Journal of General Psychology 39: 15-22

Bianchi M, Fone KF, Azmi N, Heidbreder CA, Hagan JJ, Marsden CA (2006) Isolation rearing induces recognition memory deficits accompanied by cytoskeletal alterations in rat hippocampus. European Journal of Neuroscience 24: 2894-2902

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set-shifting in the rat. Journal of Neuroscience 20: 4320-4324

Calkins ME, Gur RC, Ragland, JD, Gur RE (2005) Face recognition memory deficits and visual object memory performance in patients with schizophrenia and their relatives. American Journal of Psychiatry, 162: 1963-1966

Cilia J, Reavill C, Hagan JJ, Jones DNC (2001) Long term evaluation of isolation-rearing induced prepulse inhibition deficits in rats. Psychopharmacology 156: 327-337

Cilia J, Cluderay JE, Robbins MJ, Reavill C, Southam E, Kew JN, Jones DNC (2005) Reversal of isolation-rearing-induced PPI deficits by an alpha7 nicotinic receptor agonist. Psychopharmacology 182: 214-9

Clark RE, Zola SM, Squire LR (2000) Impaired recognition memory in rats after damage to the hippocampus. Journal of Neuroscience 20: 8853–8860.

Dalley JW, Theobald DE, Pereira EAC, Li PMMC, Robbins TW (2002) Specific abnormalities in serotonin release in the prefrontal cortex of isolation–reared rats measured during behavioural performance of a task assessing visuospatial attention and impulsivity. Psychopharmacology, 164: 329-340

Day-Wilson KM, Jones DNC, Southam E, Cilia J, Totterdell S (2006) Medial prefrontal cortex volume loss in rats with isolation rearing-induced deficits in prepulse inhibition of acoustic startle. Neuroscience 141: 1113-1121

Downes JJ, Roberts AC, Sahakian BJ, Evenden JL, Morris RG, Robbins TW (1989) Impaired extra-dimensional shift performance in medicated and unmedicated Parkinson's disease: evidence for a specific attentional dysfunction. Neuropsychologica 27: 1329-1343

Elliott BM, Grunberg NE (2005) Effects of social and physical enrichment on open field activity differ in male and female Sprague–Dawley rats. Behavioural Brain Research 165: 187-196

Elvevag B, Goldberg TE (2000) Cognitive impairment in schizophrenia is the core of the disorder. Critical Reviews in Neurobiology 14: 1-21

Ennaceur A, Delacour J (1988) A new one-trial test for neurobiological studies of memory in rats. Behavioural Brain Research 31: 47-59

Geyer M, Wilkinson L, Humby T, Robbins TW (1993) Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. Biological Psychiatry, 34: 361-372

Glantz L, Lewis D (2000) Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Archives of General Psychiatry, 57: 65-73

Grayson B, Idris NF, Neill JC (2007) Atypical antipsychotics attenuate a sub-chronic PCP-induced cognitive deficit in the novel object recognition task in the rat. Behavioural Brain Research 184: 31-38

Green MF (1996) What are the functional consequences of neurocognitive deficits of schizophrenia? American Journal of Psychiatry 153: 321-330

Green MF, Kern RS, Braff DL, Mintz J (2000) Neurocognitive deficits and functional outcome in schizophrenia: Are we measuring the “right stuff?”. Schizophrenia Bulletin 26: 119-136

Hatch AM, Wiberg GS, Zawidzka Z, Cann M, Airth JM, Grice HC (1965) Isolation syndrome in the rat. Toxicology and Applied Pharmacology 7: 737-745

Haut MW, Cahill J, Cutlip WD, Stevenson JM, Makela EH, Bloomfield SM (1996) On the nature of Wisconsin Card Sorting Test performance in schizophrenia. Psychiatry Research 65: 15-22

Jazbec S, Pantelis C, Robbins T, Weickert T, Weinberger D, and Goldberg T (2007) Intra-dimensional/extra-dimensional set-shifting performance in schizophrenia: Impact of distractors. Schizophrenia Research 89: 339-349

Jones GH, Marsden CA, Robbins TW (1990) Increased sensitivity to amphetamine and reward-related stimuli following social isolation in rats: possible disruption of dopamine-dependent mechanisms of the nucleus accumbens. Psychopharmacology 102: 364-372

Kolb B, Wilshaw IQ (1983) Performance of schizophrenic patients on tests sensitive to left or right frontal temporal, or parietal function in neurological patients. Journal of Nervous and Mental Disease 171: 435-443

Kuperberg G, Heckers S (2000) Schizophrenia and cognitive function. Current Opinion in Neurobiology 10: 205-210

Lapiz MD, Fulford A, Muchimapura S, Mason R, Parker T, Marsden CA (2003) Influence of postweaning social isolation in the rat on brain development, conditioned behavior, and neurotransmission. Neuroscience and Behavioural Physiology 33: 13-29

Lapiz MDS, Mateo Y, Parker T, Marsden CA (2000) Effects of noradrenaline depletion in the brain on response on novelty in isolation-reared rats. Psychopharmacology 152: 312-320

Li N, Wu X, Li L (2007) Chronic administration of clozapine alleviates reversal-learning impairment in isolation-reared rats. Behavioural Pharmacology 18: 135-145

Marder SR, Fenton W (2004) Measurement and Treatment Research to Improve Cognition in Schizophrenia: NIMH-MATRICS initiative to support the development of agents for improving cognition in schizophrenia. Schizophrenia Research 72: 5-9

McAlonan K, Brown VJ (2003) Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. Behavioural Brain Research 146:97-103

McLean, S, Beck, JP Woolley, ML, Neill, JC (2008) A preliminary investigation into the effects of antipsychotics on sub-chronic phencyclidine-induced deficits in attentional set-shifting in female rats. Behavioural Brain Research, 189: 152-158.

McLean SL, Shemar GK, Idris NF, Marshall KM, Neill JC (2007) Deficits in attentional set-shifting in rats following sub-chronic phencyclidine (PCP) administration, effect of gender. European Neuropsychopharmacology 17 Supp 4: S275

Pantelis C, Barber FZ, Barnes TRE, Nelson HE, Owen AM, Robbins TW (1999) Comparison of set shifting ability in patients with chronic schizophrenia and frontal lobe damage. Schizophrenia Research 37: 251-270

Puglisi-Allegra S, Oliverio A (1983) Social isolation: effects on pain threshold and stress induced analgesia. Pharmacology, Biochemistry and Behavior 19: 679-681

Sahakian BJ, Robbins TW, Iversen SD (1977) Effects of isolation rearing on exploration in rat. Animal Learning and Behavior 5: 193-198

Schrijver N, Wurbel H (2001) Early social deprivation disrupts attentional, but not affective, shifts in rats. Behavioural Neuroscience, 115: 437-442

Silva-Gomez A, Rojas D, Juarez I, Flores G (2003) Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. Brain Research, 983: 128-136

Smith JK, Neill JC, Costall B (1997) Post-weaning housing conditions influence the behavioural effects of d-amphetamine and cocaine. Psychopharmacology 131: 23-33

Sullivan EV, Shear PK, Zipursky RB, Sagar HJ, Pfefferbaum A (1994) A deficit profile of executive, memory, and motor functions in schizophrenia. Biological Psychiatry 36: 641-653

Sutcliffe JS, Marshall KM, Neill JC (2007) Influence of gender on working and spatialmemory in the novel object recognition task in the rat. Behavioural Brain Research 177: 117-125

Tyson PJ, Laws KR, Roberts KH, Mortimer AM (2004) Stability of set-shifting and planning abilities in patients with schizophrenia. Psychiatry Research 129: 229-239

Varty GB, Marsden CA, Higgins GA (1999) Reduced synaptophysin immunoreactivity in the dentate gyrus of prepulse inhibition-impaired isolation-reared rats. Brain Research 824: 197-203

Weiss IC, Pryce CR, Jongen-Relo AL, Nanz-Bahr NI, Feldon J (2004) Effect of social isolation on stress-related behavioural and neuroendocrine state in the rat. Behavioural Brain Research 152: 279-295

Tables

Table 1 Specific exemplars used (presented in pairs)

Dimension	**Pairing 1 (CD)**	**Pairing 2 (IDS)**	**Pairing 3 (EDS)**
Odour	Rose = O1 White flower = O2	Green Meadow = O3 Coconut = O4	Orange = O5 Almond = O6
Medium	Wood shavings = M1 Cat litter = M2	Small pebbles = M3 Aspen = M4	Fine sawdust = M5 Wood blocks = M6

Table 2 Time spent exploring the two identical objects during the acquisition trial of the novel object recognition task.

	Exploration time (sec)			
	Social		Isolate	
Inter-trial Interval	Left	Right	Left	Right
1-min	14.6 ± 2.6	19.3 ± 2.6	18.1 ± 2.2	18.4 ± 3.1
1-hour	12.1 ± 1.3	13.8 ± 1.5	12.3 ± 1.9	11.2 ± 1.6
3.5-hour	12.2 ± 2.1	14.5 ± 1.9	9.5 ± 1.9	11.2 ± 1.0
4-hour	10.3 ± 2.1	15.2 ± 2.8	8.8 ± 1.0	9.5 ± 1.0
5-hour	10.9 ± 2.6	9.1 ± 0.7	Not tested	Not tested
6-hour	8.8 ± 1.1	10.4 ± 1.0	Not tested	Not tested

Table 3 D1 score in retention trial of novel object recognition task

	D1 Score	
Inter-trial Interval	Social	Isolate
1-min	12.6 ± 2.3	12.9 ± 2.4
1-hour	9.7 ± 2.0	13.3 ± 2.4
3.5-hour	8.5 ± 2.4	0.5 ± 2.2 *
4-hour	8.2 ± 2.5	1.8 ± 1.5 *
5-hour	-0.6 ± 2.1	Not tested
6-hour	0.7 ± 1.2	Not tested

Table 4 Total exploration times in both trials of novel object recognition task

	Total exploration time (sec)			
	Acquisition Trial		Retention Trial	
Inter-trial Interval	Social	Isolate	Social	Isolate
1-min	33.9 ± 4.7	36.4 ± 4.6	18.9 ± 2.5	21.0 ± 3.0
1-hour	25.9 ± 2.5	23.5 ± 3.5*	20.7 ± 2.2	26.5 ± 4.9
3.5-hour	26.7 ± 3.2	20.7 ± 2.4**	24.7 ± 5.4	12.7 ± 3.1
4-hour	25.6 ± 3.7	18.3 ± 1.4**	19.8 ± 3.2	9.5 ± 1.7
5-hour	20.0 ± 3.0*	Not tested	10.4 ± 2.6	Not tested
6-hour	19.2 ± 2.0**	Not tested	13.7 ± 2.2	Not tested

Figures

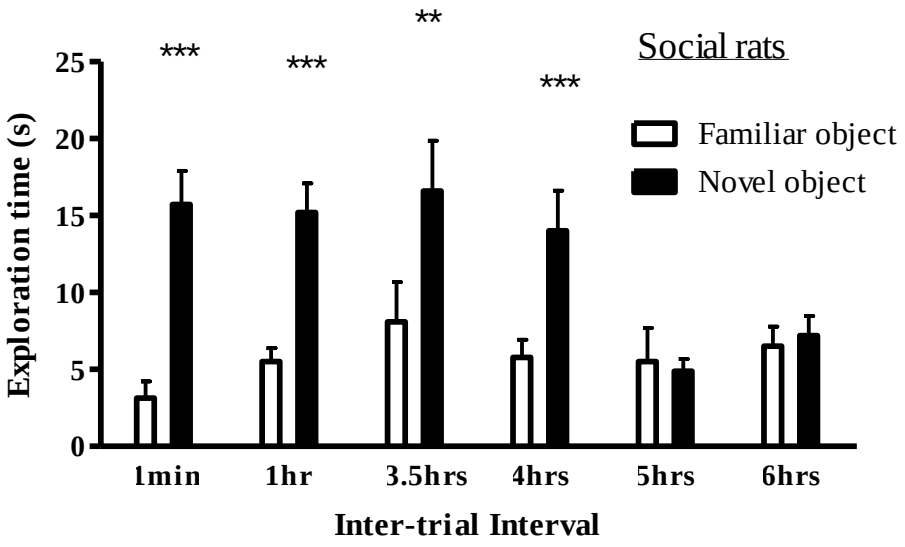

Fig. 1a

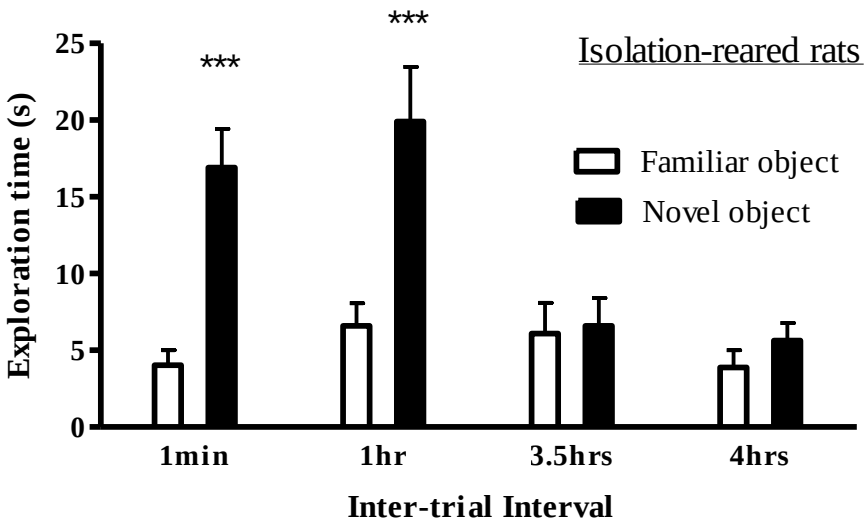

Fig. 1b

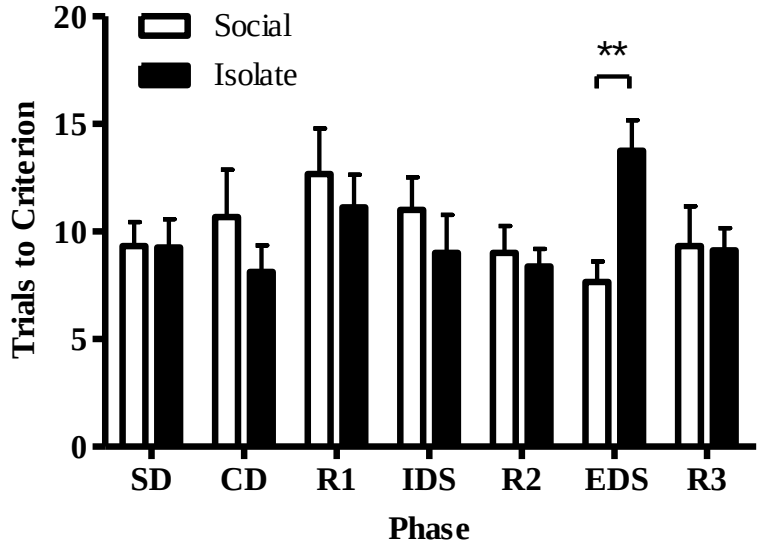
Social
Isolate
**
Trials to Criterion
20
15
10
5
0
SD
CD
R1
IDS
R2
EDS
R3
Phase

Fig. 2

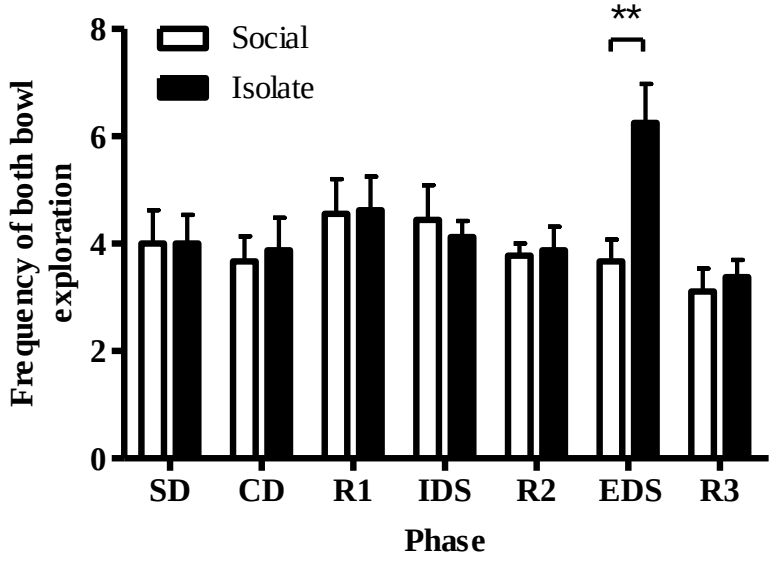
Social
Isolate
**
Frequency of both bowl exploration
8
6
4
2
0
SD
CD
R1
IDS
R2
EDS
R3
Phase

Fig. 3

Legends

Table 1: Specific exemplars used in each phase of the attentional set-shifting task. Odours were applied around the rims of digging bowls which were filled with various digging media depending upon the phase being tested. The significance of pairing within a test phase ensures that, for example, rose is always accompanied with white flower within a test trial [O = Odour, M = Medium].

Table 2: Time spent exploring the two identical objects during the acquisition trials. Data are expressed as the observed mean ± SEM (n=8-10). Both socially housed and isolated rats spent equivalent times exploring the two identical objects during the acquisition trials.

Table 3: D1 score (time exploring novel – time exploring familiar objects) in the retention trial of the novel object recognition task for social and isolated rats. Data are expressed as observed mean ± SEM (n=8-10). The D1 score was significantly reduced in isolates compared to socials at inter-trial intervals of 3.5 and 4 hours (*$p<0.05$).

Table 4: Total exploration time of social and isolated rats in the novel object recognition task in both acquisition and retention trials. Data are expressed as observed mean ± SEM (n=8-10). Total exploration time was significantly reduced when compared to the 1-min ITI in socials at 5 and 6 hour ITIs, and in isolates at 1, 3.5 and 4 hour ITIs, in the acquisition trial (**$p<0.01$, *$p<0.05$).

Figure 1a: Retention trials for the novel object recognition task in rats reared socially with increasing inter-trial intervals. Data are expressed as the observed mean ± SEM (n=8-10). Exploration time of the novel object was significantly greater than the familiar object with ITIs of 1min to 4 hours (***p<0.001, **p<0.01).

Figure 1b: The retention trials for the novel object recognition task in rats reared in isolation with increasing inter-trial intervals. Data are expressed as the observed mean ± SEM (n=8-10). Exploration time of the novel object was significantly greater than the familiar object after 1 min and 1 hour ITIs (***p<0.001).

Figure 2: The total trials to criterion in the attentional set-shifting task in isolation-reared and socially reared rats. All data expressed as observed mean ± SEM (n=9 for isolates, n=8 for socials) of total trials to criterion. Total trials to criterion was significantly greater in the isolates compared to the socials in the extra-dimensional shift phase (**p<0.01).

Figure 3: The total frequency of exploration of both bowls before digging in the attentional set-shifting task in isolation-reared and socially reared female rats. All data are expressed as observed mean ± SEM (n=9 for isolates, n=8 for socials) of frequency of exploration. Frequency of exploration of both bowls was significantly greater in the isolates compared to the socials in female rats in the extra-dimensional shift phase (**p<0.01).